METHOD FOR ENHANCING THE EFFECTIVENESS OF CANCER THERAPIES

Field of the Invention

This invention relates generally to methods and materials for the treatment of cancer. More specifically, the invention relates to methods and materials for enhancing the effectiveness of cancer therapies.

Background of the Invention

Conventional treatment for cancers involves the use of chemotherapeutic agents, radiation, and surgery, either alone or in combination. The medical arts have developed a number of treatments based upon the foregoing therapies. The present invention is directed to specific materials which can act to enhance the effectiveness of the foregoing therapies.

Galectins comprise a family of proteins which are expressed by plant and animal cells and which bind β-galactoside sugars. These proteins can be found on cell surfaces, in cytoplasm, and in extracellular fluids. They have a molecular weight in the general range of 29-34 kD; they have an affinity for β-galactoside containing materials, and have been found to play a number of important roles in biological processes including cell migration, cell-cell adhesion, angiogenesis, cell fusion and other cell-cell interactions, as well as immune-based reactions and apoptosis. As such, the role of galectins is very strongly tied to cancer and other proliferative diseases. While there are a large number of galectins which manifest the foregoing activities, galectin-3 and galectin-1 have been strongly implicated in connection with cellular processes involving cancers.

Galectin-3 is a carbohydrate binding protein having a molecular weight of approximately 30,000. It is composed of two distinct structural motifs, an amino-terminal portion containing Gly-X-Y tandem repeats which are characteristic of collagens, and a carboxyl-terminal portion containing a carbohydrate binding site. Galectin-3 is found in almost all tumors, and has a binding affinity for β-galactoside-containing glyco-conjugates. Galectin-3 is believed to play a role in mediating cell-cell interactions and thereby fostering

metastasis. It has been found that cells which have high expressions of galectin-3 are more prone to metastasis and are more resistant to apoptosis induced by chemotherapy or radiation. It has also been reported in the literature that galectin-3 plays a role in promoting angiogenesis.

Galectin-1 is a highly conserved homodimer of 14-15 kD and is one of the most abundant of the galectins. It binds to laminin which has been found to exert strong regulatory effects on cellular interactions such as adhesion, proliferation, migration and differentiation. In this regard, galectin-1 has been found to strongly influence these processes in various cells. It is believed to be implicated in the secretion of a number of cellular growth factors and interleukins. Galectin-1 has been found to be expressed at very high levels in many cancer cells and is strongly implicated in metastasis.

In accord with the present invention, it has been found that certain therapeutic materials can bind to galectins thereby inactivating them toward interaction with other carbohydrate materials and/or cells. Specifically, it has been found that treatment of galectin bearing cells with the therapeutic materials of this invention can inhibit the interaction of those cells with other cells and/or biomolecules and thereby inhibit angiogenesis and enhance the efficacy of apoptosis-inducing therapies such as chemotherapy or radiation. Furthermore, these materials can inhibit cell-cell interactions and thereby enhance the effectiveness of surgical therapies by inhibiting metastases, which are often initiated by surgical dislodgement of cells.

As will be explained in detail hereinbelow, the materials of the present invention are generally comprised of natural or synthetic polymers and oligomers. They are very low in toxicity and interact synergistically with heretofore employed cancer therapies so as to increase the effectiveness thereof. Through the use of the present invention, the dosages of potentially toxic therapies such as chemotherapies and radiation may be reduced. Likewise, the effectiveness of surgical therapies is enhanced by the use of the present invention. For example, since the methodology of the present invention acts to inhibit the post-surgery metastatic process, use of this

invention allows a surgeon to implement more aggressive surgical therapies without being limited by the possibility of precipitating metastatic events. These and other advantages of the invention will be discussed hereinbelow.

Brief Description of the Invention

There is disclosed herein a method for enhancing the efficacy of a therapeutic treatment for cancer in a patient. The treatment being enhanced may comprise chemotherapy, radiation therapy, surgery and combinations thereof. The method of the present invention comprises administering to a patient a therapeutically effective amount of a compound which binds to a galectin. This compound may be administered prior to, after, or concomitant with the other treatment.

A preferred class of therapeutic materials of the present invention comprises a polymeric backbone having side chains dependent therefrom. The side chains are terminated by a galactose or arabinose unit. This material may be synthetic, natural, or semi-synthetic. In one particular embodiment, the therapeutic compound comprises a substantially demethoxylated polygalacturonic acid backbone which is interrupted with rhamnose residues.

In general, the materials of the present invention have a molecular weight in excess of 300 dalton. One specific group of materials has a molecular weight in the range of 300 to 2,000 daltons. In those instances where the materials of the present invention are based upon complex carbohydrates such as pectins, a preferred group of materials has a molecular weight in the range of 1-50 kilodalton. The therapeutic materials of the present invention may be administered orally, by injection, transdermally, or by topical application, depending upon the specific type of cancer being treated, and the adjunct therapy.

Detailed Description of the Invention

The present invention recognizes that the effectiveness of conventional cancer therapies such as chemotherapy, surgery and radiation can be enhanced through the use of a therapeutic material which interacts with galectins.

While galectins are known to bind galactose and other such simple sugars *in vitro*, those simple sugars are not therapeutically effective in moderating galectin mediated cellular processes *in vivo*. While not wishing to be bound by speculation, the inventors hereof presume that relatively small sugar molecules are incapable of sustainably blocking, activating, suppressing, or otherwise interacting with other portions of the galectin protein. Therefore, preferred materials for the practice of the present invention generally comprise molecules which contain an active galectin binding sugar site, but which have somewhat higher molecular weights than simple sugars. Such molecules preferably have a minimum molecular weight of at least 300 daltons, and most typically a minimum molecular weight in the range of 300-2,000 daltons. Some specifically preferred materials have yet higher molecular weight ranges. A preferred class of therapeutic materials comprises oligomeric or polymeric species having one or more sugars such as galactose or arabinose pendent therefrom. The oligomeric or polymeric backbone may be synthetic or organic. Materials of this type are disclosed in U.S. Patent No. (EX SN 09/750,726) the disclosure of which is incorporated herein by reference. Such materials will preferably have a molecular weight in the range of 300-50,000 daltons and one particular material comprises a cellulose backbone with galactose terminated side chains pendent therefrom. It should be kept in mind that there is some inherent uncertainty in molecular weight measurements of high molecular weight carbohydrates, and measured molecular weights will be somewhat dependent on the method used for measuring the molecular weight. Molecular weights given herein are based on viscosity measurements, and such techniques are known in the art.

One group of materials falling within this general class comprises a substantially demethoxylated polygalacturonic acid backbone having rhamnose residues pendent therefrom. It is believed that in materials of this type, the terminal galactose or arabinose units pendent from the backbone bind to galectin proteins. The remaining bulk of the molecule potentiates the compound's action in moderating immune system response; and as discussed

hereinabove, the inventors, while not wishing to be bound by speculation, believe that the remaining bulk of the molecule either interacts with remaining portions of the galectin protein and/or prolongs the binding of the sugar portion thereto. Materials of this general type are described by formulas I, II and III hereinbelow, and it is to be understood that yet other variants of this general compound may be prepared and utilized in accord with the principles of the present invention.

$$-\left[\alpha-D-GalpA-(1\rightarrow 4)-\alpha-D-GalpA\right]_n-$$
$$\uparrow$$
$$X_{n-1} \qquad \text{(I)}$$
$$\uparrow$$
$$\alpha-L-Arap$$
$$-\left[\alpha-D-GalpA-(1\rightarrow 4)-\alpha-D-GalpA\right]_n-$$
$$\uparrow$$
$$X_{n-1}$$
$$\uparrow$$
$$\beta-D-Galp$$

where $n \geq 1$.

$$\beta-D-Galp$$
$$\downarrow$$
$$X_{n-1} \qquad \text{(II)}$$
$$\downarrow$$
$$-\left[\alpha-L-Rhap-(1\rightarrow 4)-\alpha-D-GalpA-(1\rightarrow 2)\right]_n-$$

where $n \geq 1$.

$$\alpha-L-Arap$$
$$\downarrow$$
$$X_{n-1} \qquad \text{(III)}$$
$$\downarrow$$
$$-\left[\alpha-L-Rhap-(1\rightarrow 4)-\alpha-D-GalpA-(1\rightarrow 2)\right]_n-$$

where $n \geq 1$.

Pectin is a complex carbohydrate having a highly branched structure comprised of a polygalacturonic backbone with numerous branching side chains dependent therefrom. The branching creates regions which are characterized as being "smooth" and "hairy." It has been found that pectin can be modified by various chemical, enzymatic or physical treatments to break the molecule into smaller portions having a more linearized, substantially demethoxylated polygalacturonic backbone with pendent side chains of rhamnose residues having decreased branching. This material is known in the art as modified pectin, and its efficacy in treating cancer has been established; although galectin blocker materials of this type have not been used in conjunction with surgery, chemotherapy or radiation.

U.S. Patent 5,895,784, the disclosure of which is incorporated herein by reference, describes modified pectin materials, techniques for their preparation, and use of the material as a treatment for various cancers. The material of the '784 patent is described as being prepared by a pH based modification procedure in which the pectin is put into solution and exposed to a series of programmed changes in pH which results in the breakdown of the molecule to yield therapeutically effective modified pectin. The material in the '784 patent is most preferably prepared from citrus pectin; although, it is to be understood that modified pectins may be prepared from pectin starting material obtained from other sources, such as apple pectin and the like. Also, modification processes may be accomplished by enzymatic treatment of the pectin, or by physical processes such as heating. Further disclosure of modified pectins and techniques for their preparation and use are also disclosed in U.S. Patent 5,834,442 and U.S. Patent Application Serial No. 08/024,487, the disclosures of which are incorporated herein by reference. Modified pectins of this type generally have molecular weights in the range of 1-50 kilodalton, and a preferred group of such materials has an average molecular weight in the range of 1-15 kilodalton, with a specific group of materials having a molecular weight of about 10 kilodalton.

As disclosed in the prior art, such modified pectin materials have therapeutic efficacy against a variety of cancers. These materials interact with galectins, including galectin-1 and galectin-3, and in that regard also have efficacy against immune based diseases. In accord with the present invention, the effect of conventional cancer therapies is enhanced by use of pectin materials and other materials which interact with galectins. These materials may be administered orally; or by intravenous injection; or by injection directly into an affected tissue, as for example by injection into a tumor site. In some instances the materials may be applied topically at the time surgery is carried out. Also, other techniques such as transdermal delivery systems, inhalation, subcutaneous implantation, or the like may be employed.

Radiation therapy for cancer, which includes gamma radiation as well as particle beams, and oncolytic chemotherapeutic agents are cytotoxic, and their effectiveness in treating cancer is based upon the fact that cancerous cells are generally more sensitive to such cytotoxic therapies than are normal cells either because of their rapid metabolism, or because they employ biochemical pathways not employed by normal cells. It is believed that these therapies exert their cytotoxic effects by activating programmed cell death, also referred to as apoptosis. Cells undergo apoptosis when they undergo a critical level of damage. A balance between the activities of apoptotic and anti-apoptotic intracellular signal transduction pathways is important toward a cell's decision of whether to undergo apoptosis or to attempt internal repair. It has been demonstrated that galectins, and specifically galectin-3, are involved in both apoptosis resistance and tumor progression.

Galectin-3 has been implicated in inhibiting apoptosis in cells treated with oncolytic agents such as cisplatin, genistein and the like. It was found that genistein effectively induces apoptosis, without detectable cell cycle arrest, in BT549 cells, which comprise a human breast epithelial cell line that does not express detectable levels of galectin-3. However, when galectin-3 transfected BT549 cells are treated with genistein, cell cycle arrest at the G(2)/M phase takes place without apoptosis induction (Lin et al. Galectin-3 mediates

genistein-induced G(2)/M arrest and inhibits apoptosis. *Carcinogenesis* 2000 Nov.; 21(11):1941-5). It was also found that although BT549 cells undergo anoikis, galectin-3 overexpressing BT549 cells respond to the loss of cell adhesion induced by G1 arrest without detectable cell death. Studies also suggest that galectin-3 is a critical determinant for anchorage-independent cell survival of disseminating cancer cells in the circulation during metastasis. (Kim et al. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. *Cancer Res.* 1999 Aug. 15; 59(16):4148-54).

Galectin-3 has also been shown to protect cells from apoptosis by moderating cell-cell and cell-matrix interaction, and has been shown to be involved in tumor progression and metastasis. When galectin-3 transfected human breast cancer cells are compared with their parent cell line which do not express galectin-3, it is found that the overexpressing cells: (1) had a significantly enhanced adhesion to laminin, fibronectin and vitronectin exerted both directly and/or via increased expression of specific integrins; the cells also exhibited (2) a remodeling of those cytoskeletal elements associated with cell spreading, i.e. microfilaments; and (3) enhanced survival upon exposure to different apoptotic stimuli such as cytokine and radiation (Matarrese et al. Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. *Int. J. Cancer* 2000 Feb. 15; 85(4):545-54).

The role of galectins in promoting angiogenesis has also been shown. It is known that in order for a primary tumor to grow or metastasize the cell must release chemical information instructing endothelial cells to form blood vessels which nourish and support the tumor cell. Galectins have also proven to be involved in the processes of metastasis and angiogenesis. It is shown that galectin-3 affects chemotaxis and morphology, and stimulates capillary tube formation of HUV-EC-C *in vitro* and angiogenesis *in vivo*. Endothelial cell morphogenesis is a carbohydrate-dependent process which is neutralized by specific sugars and antibodies. These findings demonstrate that endothelial cell surface carbohydrate recognition events can induce a signaling cascade leading to the differentiation and angiogenesis of endothelial cells (Nangia-Makker et

al. Galectin-3 induces endothelial cell morphogenesis and angiogenesis. *Am. J. Pathol.* 2000 Mar.; 156(3):899-909). The materials of the present invention have been demonstrated to interact with galectins and inhibit angiogenesis.

Clearly, galectins in general and galectin-3 in particular have been demonstrated to have diverse and very significant effects on the growth and proliferation of cancer cells. Furthermore, compounds which block or neutralize the activity of galectins inhibit angiogenesis and promote apoptosis. Therefore, such material will beneficially enhance the effects of oncolytic therapies. Also, it has been demonstrated that such materials will strongly inhibit angiogenesis and/or metastasis; therefore, these materials will prevent or minimize metastatic events induced by surgical disruption of a tumor site.

In accord with the present invention, a galectin binding therapeutic material is administered to a patient, in combination with conventional therapies such as surgery, radiation or chemotherapy. The material is most preferably administered prior to the administration of the conventional therapy, so as to allow it sufficient time to interact with and bind to galectins in the tumor or in non-cancerous cells. Depending on the nature of the cancer and the therapy, administration of the galectin binding therapeutic material may be continued while the other therapy is being administered and/or thereafter. Administration of the galectin binding material may be made in a single dose, or in multiple doses. In some instances, administration of the therapeutic material is commenced at least several days prior to the conventional therapy, while in other instances, administration is begun either immediately before or at the time of the administration of the conventional therapy. In some instances, particularly with regard to surgical therapies, the carbohydrate material may be advantageously administered both before, during and after the therapy.

The foregoing discussion has been primary directed toward modified pectin materials and materials which interact with galectins-1 and 3; however, it is to be understood that other galectins are also known to be involved in the progress of various cancers, and both the modified pectin material as well as

the other therapeutic materials discussed hereinabove interact with galectins. Therefore, other materials and methods may be employed in the practice of the present invention. The foregoing discussion and description is illustrative of specific embodiments, but is not meant to be a limitation upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

Claims

1. A method for enhancing the efficacy of a therapeutic treatment for cancer in a patient, said therapeutic treatment being selected from the group consisting of: chemotherapy, radiation therapy, surgery, and combinations thereof, said method comprising the steps of:

administering to said patient a therapeutically effective amount of a compound which binds to a galectin; and

administering said therapeutic treatment to said patient.

2. The method of claim 1, wherein said galectin is present on the cell surface of a tissue of said patient.

3. The method of claim 1, wherein said compound binds to galectin-1 or galectin-3.

4. The method of claim 1, wherein said compound comprises a polymeric backbone having side chains dependent therefrom, said side chains being terminated by a galactose or arabinose unit.

5. The method of claim 1, wherein said compound comprises a substantially demethoxylated polygalacturonic acid which is interrupted with rhamnose residues.

6. The method of claim 1, wherein said compound comprises a carbohydrate.

7. The method of claim 6, wherein said carbohydrate comprises a branched carbohydrate.

8. The method of claim 1, wherein said compound comprises a modified pectin.

9. The method of claim 8, wherein said modified pectin comprises a pH modified pectin.

10. The method of claim 9, wherein said modified pectin comprises an enzymatically modified pectin.

11. The method of claim 8, wherein said modified pectin comprises a thermally modified pectin.

12. The method of claim 8, wherein said modified pectin comprises a modified citrus pectin.

13. The method of claim 1, wherein said compound has a molecular weight of at least 300 dalton.

14. The method of claim 1, wherein said compound has a molecular weight in the range of 300-2,000 dalton.

15. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-50 kilodalton.

16. The method of claim 8, wherein said modified pectin has a molecular weight in the range of 1-15 kilodalton.

17. The method of claim 8, wherein said modified pectin has a molecular weight of approximately 10 kilodalton.

18. The method of claim 1, wherein said step of administering said compound to said patient comprises injecting said compound into said patient.

19. The method of claim 1, wherein said step of administering said compound to said patient comprises orally administering said compound to said patient.

20. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound prior to administering said therapeutic treatment to said patient.

21. The method of claim 1, wherein said step of administering said compound to said patient comprises administering said compound to said patient after said therapeutic treatment is administered to said patient.

22. The method of claim 1, wherein said compound is administered concomitant with said therapeutic treatment.